PRODUCT SPECIFICATION SHEET

DM494-Modified Rogosa Agar (M16 Agar) (DM494)

Intended Use

M16 Agar is recommended for cultivation and enumeration of lactic streptococci used in manufacture of cheddar cheese.

Product Summary and Explanation

Selective inhibitory effects of selenite were first demonstrated by Klett.[1] Guth[2] used it to isolate *Salmonella typhi*. Leifson[3] found that selenite inhibited fecal streptococci and coliforms during the first 8-12 hours of incubation, thereby permitting salmonellae to replicate without overwhelming interference from other members of the intestinal flora. North and Bartram[4] modified Leifson's Selenite-F Enrichment broth by adding cystine, which stimulated growth of *Salmonella*. The cystine-containing formulation is recommended by the Food and Drug Administration, AOAC International and American Public Health Association for detecting *Salmonella* in foods, particularly egg products and waters.[5-8] It is also recommended by APHA and USP.[9, 10] Selenite Cystine Broth is useful for detecting *Salmonella* in the non-acute stages of illness when organisms occur in the faeces in low numbers and for epidemiological studies to enhance the detection of low numbers of organisms from asymptomatic or convalescent patients.[11] *Salmonella* are also injured during various food processing procedures, including exposure to low temperatures, submarginal heat, drying, radiation, preservatives or sanitizers.[12] Since, *Salmonella* may be present in low numbers in food sample in an injured condition; recovery of *Salmonella* involves pre-enrichment, selective enrichment and selective plating. Fluid Selenite Cystine Medium is used as selective enrichment medium for the cultivation of *Salmonella* species. This medium is formulated to allow the proliferation of *Salmonella* while inhibiting the growth of competing non-*Salmonella* organisms.

Principles of the Procedure

A variety of acid-producing bacteria are found in nature, in the soil, on raw agricultural products and in certain processed foods. One of the most important groups of acid producing bacteria in food industry is the lactic acid bacteria. Streptococci belong to the lactic acid bacteria group. Streptococcus is a genus of spherical, gram-positive bacteria, and a member of the phylum Firmicutes (1). M16 Agar is a modification of Rogosa Sodium Lactate Agar recommended by APHA (1, 3). This medium was developed to support growth of lactic streptococci used in cheddar cheese manufacturing in New Zealand (2). This medium can also be used as selective medium for the cultivation of oral and faecal lactobacilli. Since some lactobacilli do not grow on this medium if incubated aerobically, incubation in a CO2-enriched atmosphere is recommended. The large number of media proposed for lactic acid bacteria, particularly for streptococci and /or lactobacilli, is an indicative of the variability in growth features of different species, thereby the difficulties encountered in growing some strains of this group of organisms. While the lactic acid bacteria in general are tolerant to low pH, they can be very sensitive to other adverse conditions. Freezing and thawing prior to analysis may be detrimental to cell growth. Dilution process may also damage lactic acid bacteria in samples, thus it is best to use sterile 0.1% Peptone Water (M028) as the diluent. Papaic digest of soyabean meal, tryptose and beef extract provide the essential nutrients like amino acids, minerals etc. Yeast extract supplies vitamin B complex to the lactic streptococci. Dextrose is the fermentable carbohydrate and energy source. Sodium acetate inhibits other contaminating bacteria and suppresses swarming growth. Ascorbic acid provides vitamin C to the organisms. The samples to be tested are processed to enumerate bacteria by pour plate technique

Formula / Liter

Ingredients	Gms / Liter
Papic digest of soyabean meal	5.000
Tryptose	5.000
Beef extract	5.000
Yeast extract	2.500
Dextrose	5.000
Ascorbic acid	0.500
Sodium acetate	3.000
Agar	10.000
Final pH: 7.2 ± 0.2 at 25°C Formula may be adjusted and/or supplemented as required to meet performance specifications	

Precautions

In Vitro diagnostic Use only. Read the label before opening the container. Wear protective gloves/protective clothing/ eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Directions

1. Suspend 36 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely.
2. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well and pour into sterile Petri plates.

Quality Control Specifications

Dehydrated Appearance	Cream to yellow homogeneous free flowing powder
Prepared Medium	Light amber coloured clear to slightly opalescent gel forms in Petri plates
Reaction 3.6% solution	pH 7.2 ± 0.2 at 25°C
Gel Strength	Firm, comparable with 1.0% Agar gel

Expected Cultural Response: Cultural characteristics observed after an incubation at 35-37°C for 18-48 hours in CO2 enriched atmosphere.

Sr. No.	Organisms	Results to be achieved		
		Inoculum (CFU)	Growth	Recovery
1.	Lactobacillus lactis ATCC 8000	50-100	good-luxuriant	>=50%
2.	Streptococcus cremoris ATCC 19257	50-100	good-luxuriant	>=50%

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Prepare food sample following the recommended procedure.
2. Inoculate into recommended pre-enrichment broth.
3. Transfer 1 mL of mixture to 10 mL Selenite Cystine Broth and to 10 mL Tetrathionate Broth.
4. Incubate at 35°C for 24 ± 2 hours.
5. Mix and streak 3 mm loopful (10 µL) of sample from both broths onto Bismuth Sulfite Agar, Xylose Lysine Desoxycholate Agar, Hektoen Enteric Agar or MacConkey Agar.
6. Examine plates for the presence of colonies that are typical for *Salmonella* spp.
7. Refer appropriate references for standard test procedures.

Results

Refer appropriate references and procedures for interpretation of results.

Storage

Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1.The Bacterias sustaining low pH, may grow on this media.
2. Some organisms may show poor growth due to nutritional variations.

Packaging
Product Name : DM494-Modified Rogosa Agar (M16 Agar) (DM494)
Product Code : DM494
Available Pack sizes : 100gm / 500gm

References

1. Downes F. P. and Ito K., (Eds.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th Ed., APHA, Washington, D.C.
2. Lowrie R. J. and Pearce L. E., 1971, New Zealand, J. Dairy Sci. Technol., 6: 166.
3. Rogosa M., Mitchell J. A. and Wiseman R. F., 1951, J. Bacteriol., 62 : 132-133

Further Information

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